

Claims

1. A method of generating electrocompetent cells, said method comprising:
 - a) growing bacterial cells in culture medium at hyperosmotic salt concentration ; and
 - b) treating said cells to make them electrocompetent.
- 5 2. The method of claim 1 wherein said electrocompetent cells have an electrotransformation efficiency at least 30% greater than that for cells of the same bacterial strain grown under conditions of isoosmotic salt.
3. The method of claim 1 wherein said electrocompetent cells have an electrotransformation efficiency of at least 2×10^{10} cfu/ μ g DNA.
- 10 4. The method of claim 1 wherein said bacterial cells are Gram negative cells.
5. The method of claim 1 wherein said bacterial cells are E. coli cells.
6. The method of claim 1 wherein said hyperosmotic salt concentration is 100 mM to 350 mM above isoosmotic.
7. The method of claim 1 wherein said hyperosmotic salt concentration is 150 mM to 225
15 mM above isoosmotic.
8. The method of claim 1 wherein said hyperosmotic salt concentration is 200 mM above isoosmotic.
9. The method of claim 1, wherein said step of growing bacterial cells at hyperosmotic salt concentration further comprises growing said cells under conditions of limited dissolved oxygen
20 concentration.
10. The method of claim 9 wherein said conditions of limited dissolved oxygen concentration comprise a 1 to 10-fold reduction in dissolved oxygen relative to cultures grown under conditions of maximal aeration.
11. The method of claim 1 wherein step (b) comprises contacting said cells with glycerol.

12. The method of claim 1 wherein said cells are contacted with a 10% solution of glycerol in water.
13. The method of claim 12 wherein said 10% solution of glycerol in water further comprises sorbitol.
- 5 14. The method of claim 1 further comprising the step of drying said electrocompetent cells.
15. The method of claim 14 wherein upon re-hydration, the viable cells remain electrocompetent.
16. The method of claim 1 wherein step (a) comprises growing said bacterial cells to a final OD₅₅₀ of 0.45 to 0.5.
- 10 17. The method of claim 1 wherein said culture medium comprises casein hydrolysate and/or maltose.
18. The method of claim 17 wherein said casein hydrolysate is present in said culture medium at a concentration of 11-15 g/liter.
19. The method of claim 17 wherein said casein hydrolysate is present in said culture
15 medium at a concentration of 11-12 g/liter, inclusive.
20. The method of claim 17 wherein said maltose is present in said culture medium at a concentration of 0.1-0.3 % (w/v).
21. The method of claim 17 wherein said maltose is present in said culture medium at a concentration of 0.2-0.3% (w/v), inclusive.
- 20 22. A method of producing a transformed cell, said method comprising
- a) obtaining electrocompetent cells generated according to the method of claim 1;
 - b) mixing said electrocompetent cells with a nucleic acid encoding said recombinant polypeptide;
 - c) subjecting the mixture of step (b) to an electrical treatment; and

d) culturing said cells, such that a transformed cell is produced.

23. A method of producing a recombinant polypeptide comprising:

a) obtaining electrocompetent cells generated according to the method of claim 1;

5 b) mixing said electrocompetent cells with a nucleic acid encoding said recombinant polypeptide;

c) subjecting the mixture of step (b) to an electrical treatment; and

d) culturing said cells in a cell growth medium under conditions in which the cells produce said polypeptide.

10 24. The method of claim 23, in which cells which have taken up said nucleic acid are separated from cells which have not taken up said nucleic acids.

25. The method of claim 23, wherein said recombinant polypeptide is isolated from said cells.

15 26. A biologically pure E. coli culture having all identifying characteristics of the E. coli strain 209K15 deposited with the American Type Culture Collection (ATCC) and assigned Accession No. PTA-5025, or mutants thereof that maintain increased transformation efficiency relative to the E. coli strain of ATCC Accession No. PTA-369.

27. An electrocompetent cell according to claim 26.

28. A viable dried cell according to claim 26.

29. The cell of claim 27 that is electrocompetent upon re-hydration.